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Role of the Dosage-Response Curve In the Evaluation of Fungicides

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CONTENTS

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WITHIN the past ten years, fungicidal activity for a large number of compounds has been measured systematically in the laboratory. Laboratory tests have been so refined that it is now possible to isolate and study quantitatively many of the variables that affect the performance of fungicides.

As a result of the quantitative approach, a number of old concepts have had to be modified and a few new ones have appeared. It is the purpose of this paper to present some of these and to discuss their implications. The argument is presented that the concepts, developed in the laboratory, should be valid in the field when properly modified, and that the design for field experiments might profit from the experience gained in the laboratory.

MATERIALS AND METHODS

Laboratory

The method of testing fungicides in this laboratory has been described (16). Stock suspensions of toxicant were prepared by dispersing weighed samples of toxicant in known volumes of water. While being stirred, this stock was sprayed by means of a deVilbiss atomizer through a sewer tile (13) on to a glass microscope slide coated with cellulose nitrate. The glass slides were then dried. The amount of toxicant deposited per square centimeter of slide surface is determined from the rate of spray fluid deposition, spraying time, and the concentration of the stock suspension.

The test organism was *Macrosporium sarcinaeforme* Cav., grown for 19 to 21 days on oat meal agar at 20° C. Spores were washed from the slants with double-distilled water and the spore load was adjusted so that the third drop from a standardized one ml. pipette contained 40 spores per low power microscopic field (10 × ocular — 16 mm. objective).

Two drops of spore suspension were dropped on each dried slide. The slides were then placed on aluminum slide racks and

[1]The authors would like to express their best thanks to Dr. C. I. Bliss, Biometrician, and Mr. Neely Turner, Assistant Research Entomologist, for their suggestions and criticisms of this manuscript.

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incubated in moist chambers for 15 hours at 20° C. Spore inhibition was determined from fifty viable spores in each of the two drops per fungicide dosage.

The methods employed in the radiation studies were those used by Dimond and Duggar (8). *Rhizopus suinus* Niels. was the test organism. Spores were obtained from cultures grown on a peptone-dextrose nutrient. After irradiation, they were incubated at 28° C. in hanging drops in a potato-dextrose nutrient. Each experimental point is based upon 600 spores (300 per hanging drop, two drops per dosage of ultraviolet).

Field

Data on apple scab were obtained from McIntosh trees on the Station Farm at Mount Carmel, Conn. Five sprays of a wettable sulfur were applied with a broom type nozzle at 400 pounds pressure. These sprays included a prepink spray, a pink, a calyx, and two cover sprays. The first cover spray was applied 10 days after the calyx spray and the second, two weeks later. Each point is based upon the reading of 400 leaves from randomized trees.

Tomatoes were sprayed with a power sprayer at 250 pounds pressure. Sprays were applied at weekly intervals from July 15 onward. Each experimental point is based upon 40 plants in four replicates, randomized in blocks. Celery was sprayed by knapsack sprayers at weekly intervals. Each point is based on 60 plants in four replicates, randomized in blocks.

Foliage was scored by setting up five classes representing the scale of disease magnitude, the lowest class being 0 and the highest 4, according to the method recently described (14). In the case of the tomatoes, the plant as a whole was judged in terms of the percentage defoliation which the fungus had induced. For celery, the entire plant was harvested and each leaf was scored for its disease rating. When the plot had been rated, the number of leaves in each class was multiplied by its class number. These were summed for the five classes. This figure was then divided by the total number of leaves examined to obtain an index of disease for the treatment as a whole. These indices were converted to percentage of disease by establishing the ratio between the index obtained and the maximum possible index, 4.00. Subtraction of the percent disease from 100 converted the values to percent disease control.

EVALUATION OF FUNGICIDES IN THE LABORATORY

The Dosage-Response Curve

The first procedure in estimating toxicity[1] is the plotting of a dosage-response curve. When percent inhibition is plotted against

[1]Materials may inhibit fungous spores through fungicidal or fungistatic action. The first type of action involves death of spores whereas the second involves only inhibition. These two types may be distinguished by observing whether or not spores of a population exposed to a lethal dose of toxicant will germinate on removal of toxicant after a certain exposure period has elapsed.

Whether the response is the result of inhibition or death of spores, the principles involved in measuring the level of toxicity for a given material are the same, as also are the methods for comparing one toxicant with another.

dosage of toxicant, the resulting curve usually is sigmoid in shape (Figure 1a). This relationship has long been known and various papers have been written on the forces determining the shape of this curve. Among biologists, a favored explanation is that the variation in resistance among spores, as measured by the logarithm of the threshold dosage of toxicity, follows the normal distribution. Gaddum (11) and Bliss (1) first showed such a relation to hold in toxicity studies. Wilcoxon and McCallan (22) applied these ideas to fungicide tests, and suggested that the use of logarithmic-probability coordinates would permit an investigator to plot percent response directly against dosage of toxicant to obtain straight lines[1] (Figure 1b).

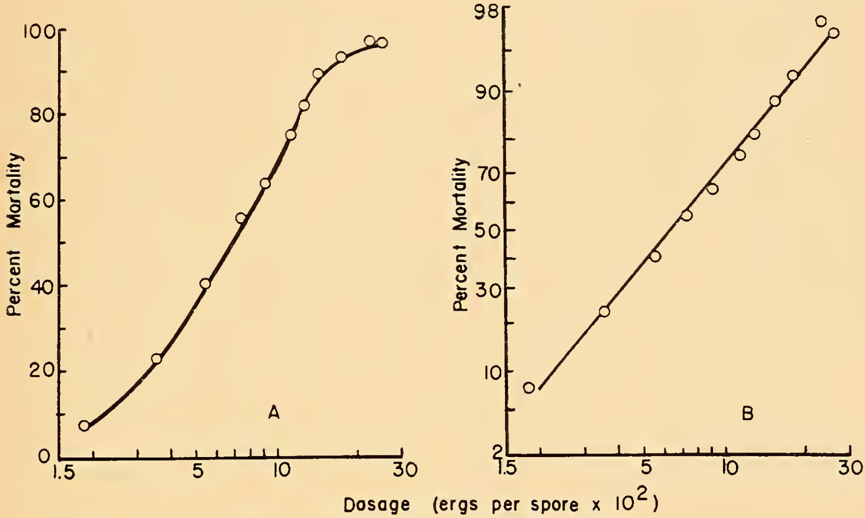

FIGURE 1. The conversion of dosage-response curves from sigmoid to linear functions by means of the logarithmic-probability grid. Fig. 1a: the sigmoid relation between dosage of monochromatic ultraviolet radiation (2650 A.) and mortality to spores of *Rhizopus suinus*, when data are plotted on arithmetic cross-section paper. Fig. 1b: the linear relation between these factors on the logarithmic-probability grid.

The conversion of dosage-response curves from sigmoid shape to straight lines has been a contribution of considerable consequence. Advantages of a straight line over a sigmoid relation are threefold: (1) Straight lines can be more readily and accurately interpolated and extrapolated than can sigmoid curves. (2) LD-50 values (the dosage required to inactivate 50 percent of a spore population) are

[1]There are a number of methods by which sigmoid dosage-response curves can be converted to straight lines. Statisticians are not in complete agreement as to which method is most sound for effecting this conversion. While the interpretation may vary, the effect of the factors to be considered upon the performance of fungicides does not appear to depend upon the method of conversion employed.

more readily and accurately determined. (3) The slope of the dosage-response curve can be determined. This was not previously possible. LD-50 and slope represent two fundamental properties of fungicides.

THE LD-50

Definition. The quantity of fungicide required to inhibit a fixed percentage (usually 50 percent) of fungous spores has been used for some time as an index of toxicant potency. Its numerical value depends on a number of factors. It is affected not only by the fungicide, but also by the fungous species used in the test (17), the spore load (17), age of the spores (18), size of the spore drop (16), environmental factors, and slope of the dosage-response curve.

In comparing one fungicide with another, one should bear in mind that the abscissa of the dosage-response curve is logarithmic, and that the comparison of fungicides should allow for this.

Factors affecting LD-50. The resistance level of spores of different fungous species appears to vary over a wide range (17). This variation in resistance may be correlated with the volume of the spore (9, 15), shape of the spore, the surface exposed, the number of cells which the spore possesses ,the number of nuclei per spore (9), etc. In any case, when more than one fungous species is used in testing toxic action, toxicants should be compared from their relative action on a single species.

Spore load is one of the factors giving rise to variation in LD-50 values. McCallan, Wellman, and Wilcoxon (17) have shown that the logarithm of the LD-50 value varies in direct proportion with the logarithm of the spore load. Our own experiments have confirmed this relationship.

The age of the spores used has definite bearing upon the value of the LD-50 obtained. For ultraviolet radiation, Oster (19) has found for yeast an increased resistance to killing with age of culture. This result has been confirmed by Dimond and Duggar (9) for the fungus *Rhizopus suinus*.

On the other hand, the resistance of *Macrosporium sarcinaeforme* to the so-called insoluble copper compounds appears to decrease with age (18). It would appear as though resistance may vary in either possible direction with age, but, in any case, LD-50 values will be governed in part by the age of the culture used.

Control of environmental factors is important in stabilizing LD-50 values. It is well known that spores are more difficult to inhibit by fungicides when they are at their optimum than when they are subjected to an unfavorable environment, *i.e.*, the LD-50 is higher. For example, in one experiment, more zinc oxide was required for 50 percent inhibition of *Macrosporium* spores at 20° C. than at 30° C., because 30° C. is above the optimum temperature.

The influence of the medium in which spores were germinated was tested in another experiment. In the presence of red cuprous oxide as a fungicide, spores of *Macrosporium sarcinaeforme* were germinated in double-distilled water in one case, and in 0.1 percent filtered orange juice in another (Figure 2).

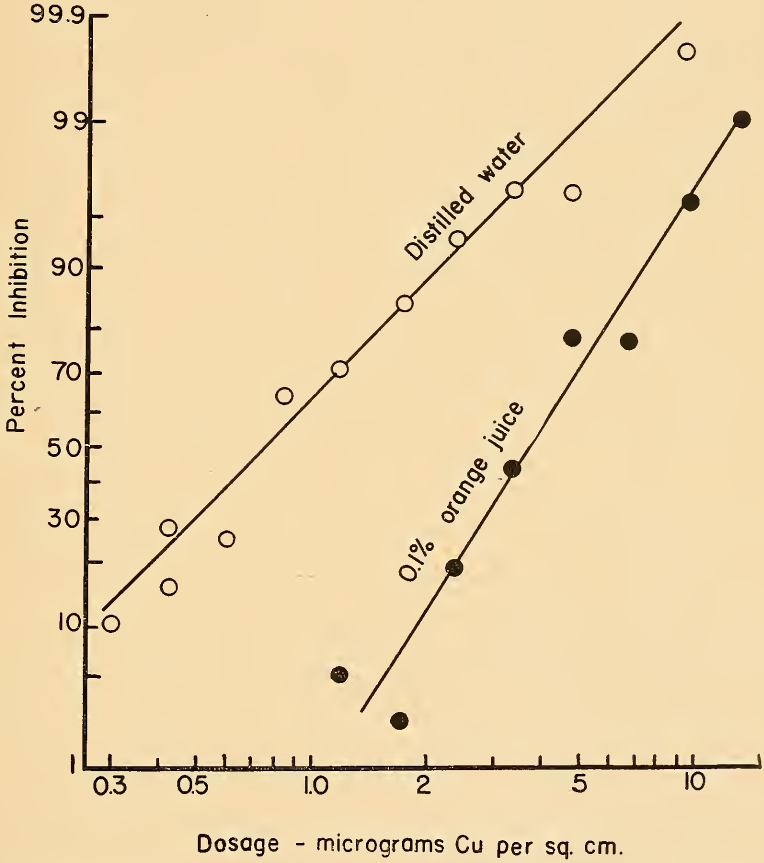

FIGURE 2. The effect of the medium in which spores are germinated on the value of the LD-50. Fungicide: red cuprous oxide. Fungus: *Macrosporium sarcinaeforme.* Variable: nutritional conditions. Curve 1: spore germination in distilled water; curve 2: spore germination in 0.1 percent filtered orange juice.

In this experiment, the same fungus required five times more copper in red cuprous oxide for 50 percent inhibition when germinated in the orange juice than when germinated in distilled water at 20° C.

The effect of slope of the dosage-response curve on variation of LD-50 will be described below.

SLOPE OF THE DOSAGE-RESPONSE CURVE

Significance and measurement. Perhaps the most important advantage of the use of the logarithmic-probability grid for toxicity data is that the slope of the dosage-response curve can be determined. If LD-50 is a measure of spore-inhibiting properties of a toxicant at one level of inhibition, slope of the dosage-response curve measures the spore-inhibiting properties of a toxicant under conditions of changing dosage. Statistically, a steep slope indicates that the spore population behaves more uniformly than when the dosage-response curve is flat. Slope may be defined as the increase in mortality for a constant percentage increase in dosage of fungicide. More exactly, inhibition is first transformed to probability units and dosage to logarithms. Bliss (2) has computed a table for conversion of percent inhibition to probability (standard deviation) units which he has called probits. The unit of slope is in mortality probits per log dose.

The importance of slope as a property of the fungicide has only recently been realized, and is just beginning to be measured in tests of fungicidal potency. Yet, in many respects, slope is as important an aspect of toxic action as is LD-50. It can be evaluated quite simply from the data obtained to determine LD-50.

Some sort of index which combines in one number an evaluation of LD-50 and slope of the dosage-response curve is needed in fungicidal ratings. Such a rating will reflect many more properties of the toxicant than does LD-50 or the Bordeaux coefficient, which dimensionally is identical with LD-50.

Factors affecting slope of the dosage-response curve. The slope of the dosage-response curve may vary with the fungus used in tests, though not necessarily so. Slope is also dependent upon the fungicide being tested, and under standardized conditions is as much a property of the fungicide as LD-50.

The age of the culture used as a source of spores alters the value of the slope of the dosage-response curve. In an experiment testing this point, spores from 14- and 21-day cultures of *Macrosporium sarcinaeforme* were exposed to copper silicate, and two dosage-response curves were obtained. The curve for the older spores, in addition to having lower LD-50 values, has a flatter slope than the curve for spores from the 14-day culture (Figure 3). These results were confirmed for a number of other copper fungicides, including Bordeaux mixture. The dosage-response curve of the old spores was always flatter than that of the young spores and converged with it at high inhibition levels.

Evidently, then, spores become less resistant to copper as they grow older. This appears to result from the fact that the slope of

the dosage-response curve decreases as spores become older and is an illustration of the change in LD-50 values brought about by a change in slope of the dosage-response curve.

Environmental factors which vary during the period of test or from one test to the next may lead to variation in slope of the dosage-response curve. Figure 2 shows that the slope of the dosage-response curve of red cuprous oxide is steeper when the spores of

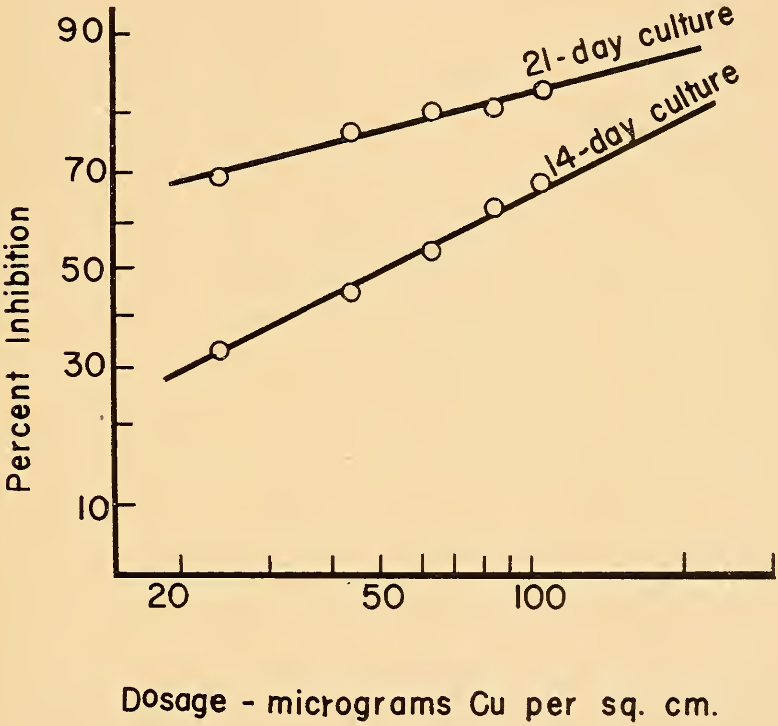

FIGURE 3. The influence of age of the culture used as a source of spores on the slope of the dosage-response curve. The fungicide is copper silicate and the fungus is *Macrosporium sarcinaeforme.*

the fungus *Macrosporium sarcinaeforme* are germinated in 0.1 percent filtered orange juice than when they are germinated in distilled water. In general, it would appear as though any factor which brings the fungus nearer its optimum for germination causes an increase in the slope of the dosage-response curve, and that the slope of the curve is greatest when the fungus is at its optimum. Temperature, the type of nutrient, and accessory growth factors present in the spore drop may all be expected to give rise to this type of variation.

The time at which counts of spore inhibition are made appears to be an exceedingly important factor in determining the slope of the dosage-response curve (10), and, secondarily, of the LD-50 (21). Dimond and Duggar (8), in a study of ultraviolet effects on the germination of fungous spores, made successive counts of germination of spores exposed to several dosages of ultraviolet. Their data have been recomputed and are presented in Figure 4. The family

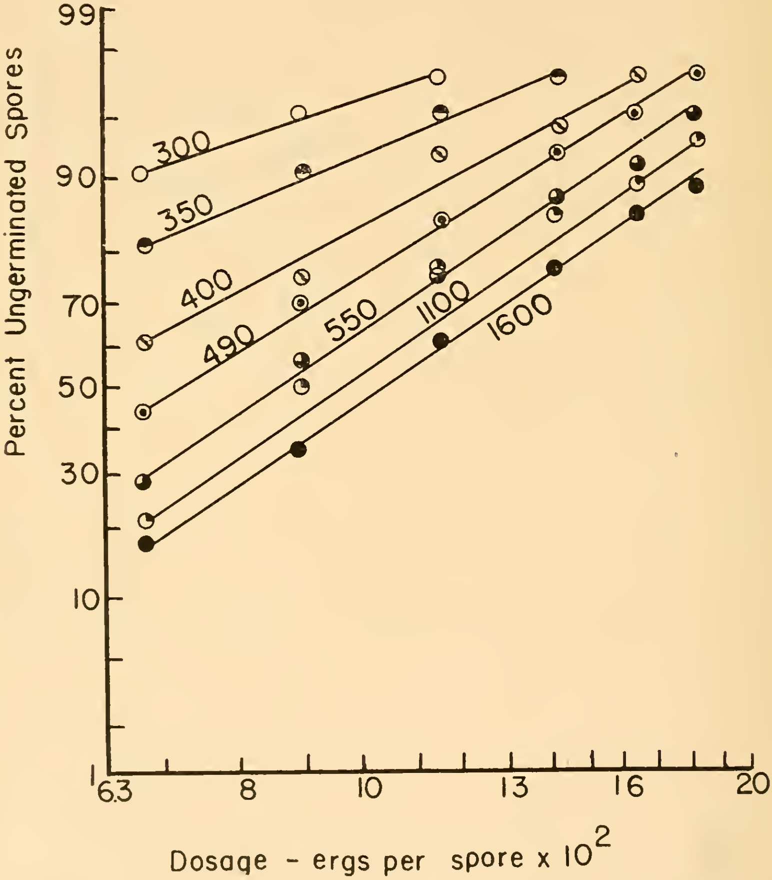

FIGURE 4. The influence of incubation time on the properties of the dosage-response curve. Numbers on curves indicate time of incubation in minutes.

of curves obtained indicates that the slope of the dosage-response curve increases until the process of germination has become complete. The reciprocal of the slope of these dosage-response curves λ) decreases in value with time, and gradually becomes constant (Figure 5).

It is clear that early counts will tend to underestimate both slope and LD-50 of a given toxicant. Underestimation of LD-50 results in an overestimation of toxicity; the underestimation of slope under the proper circumstances may lead to a similar error.

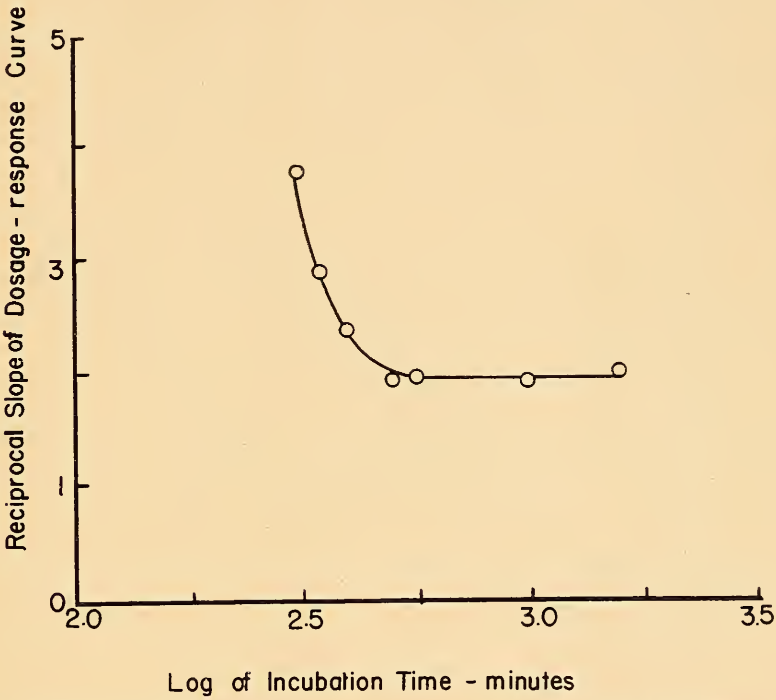

FIGURE 5. The relation between reciprocal slope of the dosage-response curve (λ) and logarithm of incubation time.

Although difficult to demonstrate for all fungicides in the laboratory, it can be shown in certain cases that slope of the dosage-response curve depends for its value upon the type of coverage of fungicide over the slide surface. Such experiments are more readily conducted under field conditions, and experiments demonstrating this point will be described in the section dealing with the application of the dosage-response curve to field data.

The spore load used in fungicide tests, and the concentration of the stock of fungicide employed appears to have no effect on the slope of the dosage-response curve. In an experiment with copper oxychloride, in which concentration of the stock of fungicide and the spore load each were varied, no change in slope of the dosage-response curve was observed (Figure 6). This important point should be borne in mind in the discussion of field uses of the dosage-response curve.

LD-50 VERSUS LD-95

The field pathologist justifiably wonders why LD-50 is chosen as a comparison point for measuring the spore-inhibiting properties of a toxicant, rather than a higher level of inhibition, such as LD-95 or LD-99. The chief advantage of LD-50 is that it can be located with the greatest precision in experimental work for a spore population of given size. LD-95 could be determined with as great precision as LD-50 only if a greater number of spores were counted in toxicity tests or if a greater number of tests were made for each toxicant.

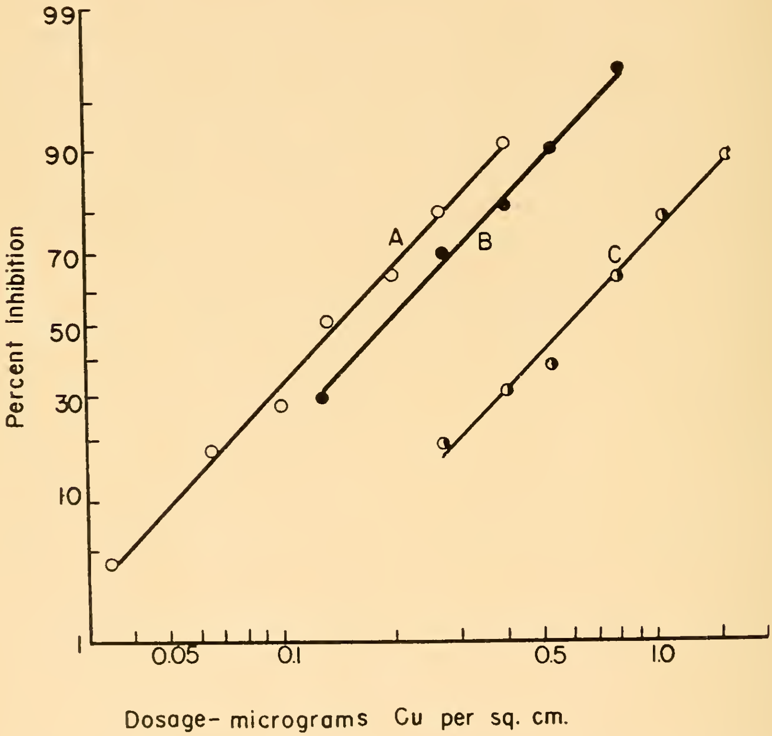

FIGURE 6. The effect of stock concentration of fungicide and of spore load on the slope of the dosage-response curve. Fungicide: copper oxychloride (Grasselli Copper Compound A). Curve A: 20 spores per field, stock of fungicide 0.025 percent copper. Curve B: 20 spores per field, stock of fungicide 0.1 percent copper. Curve C: 80 spores per field, stock of fungicide 0.1 percent copper.

LD-95, on the other hand, is a level of spore inhibition of practical value to the grower and involves no extrapolation for its evaluation.

Another advantage in the use of LD-95 as compared with LD-50 is coupled with the fact that time allowed for germination to occur and changes in environmental factors during the period of the test both tend to affect the slope of the dosage-response curve (Figures 2, 3 and 4). The curves of these figures all tend to converge at some point near the 100 percent level of inhibition. It can be readily seen that LD-95 shows much less variability than does LD-50 owing to this convergent tendency. Consequently, errors arising from making mortality counts too soon, and variations in slope caused by environmental and nutritional factors would be minimized if LD-95 were chosen rather than LD-50.

Types of Dosage-Response Curves

STRAIGHT LINES

The foregoing discussion has indicated a number of factors which must be held constant in order that LD-95 and slope values shall be characteristic of the fungicide. In the remaining discussion of this section of the paper, references to LD-50 and slope values will be made with the assumption that they are properties of the fungicide itself.

Toxicants vary widely in their spore-killing properties. Either LD-95 or slope may vary, and presumably they vary independently of one another. Four types of dosage-response curves, basically, will cover the range of possibilities (Table 1).

TABLE 1. THE EXTREME TYPES OF LINEAR DOSAGE-RESPONSE CURVES

		Slope	
		low	high
LD-95[1]	low	*a*	*b*
	high	*c*	*d*

A fungicide of *type a* characteristically has low values of LD-95 and slope. Materials having such dosage-response curves are rare and none has yet been used in our field experiments, although they perform well in the laboratory. Materials of *type b*, with low LD-95 and steep slope, are well known and in common use, Bordeaux mixture being an example. Bliss (4) has suggested that materials having these properties are preferable to materials having flat-sloped curves. If materials were compared through their LD-50 values, this would be true, but when they are compared at the LD-95 level, materials of *type a* may prove superior to those of *type b*.

The reason for suggesting steepness of slope as a desirable property (4) is that a small dosage increment leads to a considerable increment in spore inhibition when the toxicity curve is steep. For the action of drugs on experimental animals, this is a prime consideration, but for the action of protectant fungicides on plants, another factor must be considered, *viz.*, tenacity of the material to the

[1]LD-95 is chosen for this comparison rather than LD-50 because fungicides of *type a* stand out favorably in contrast with those of *type b* only when they are compared at high levels of comparable control.

plant surface (12). As a matter of fact, all factors in the field tend to decrease the dosage of toxicant applied to the plant. Rain, wind, and the accumulation of dirt on the foliage of the plant tend to make less available or to remove the deposit of toxicant from the leaf surface. This being the case, a fungicide having a dosage-response curve with a steep slope is markedly reduced in spore-inhibiting power by a 50 percent reduction in dosage. Another material, possessing a flat dosage-response curve, could undergo the same reduction in dosage with much less loss in spore inhibiting power.

As an example, let it be assumed that two fungicides are being compared, one of *type a* and the other of *type b*, and that the tenacity of these materials is the same (Figure 7). These materials are applied to the leaf surface at their LD-95 values in each case. Then, if weathering occurs so that the dosage for each compound is reduced to half of its initial value, it is apparent that a fungicide of *type a* would be capable of 91 percent disease-control after weathering, whereas a material of *type b* would be capable of only 34 percent disease control (Figure 7).

Clearly, there is a relation between the slope of the dosage-response curve, as determined in the laboratory, and the *importance* of tenacity; the steeper the dosage-response curve, the higher the tenacity must be in order that a material belonging to *type b* will perform well in the field. In support of this argument is the fact that Bordeaux mixture and copper oxychloride (Grasselli Copper Compound A) are protectant fungicides belonging to *type b*. Bordeaux mixture has a very high tenacity whereas copper oxychloride has not. This difference in tenacity is in line with the differences in disease-controlling power between these materials.

A material of *type a* desirably should have high tenacity, but need not have the tenacity essential to a material with properties of *type b*. The inference must not be drawn, however, that the slope of the dosage-response curve, determined in the laboratory, is in any sense a measure of tenacity; this is not the case.

When the tenacity is high, it is true that a material of *type b* can be applied to the plant so as to overcome the disadvantage of a steep slope. If applied far in excess of the LD-95, sufficient material will remain so that the dosage of toxicant will never be reduced to the LD-95 level. This involves the cost factor and can be used as a procedure only if the material is relatively cheap.

Materials of *types c* and *d* represent the other extremes of fungicidal activity. A material of *type c* is only a fair fungicide, whereas one of *type d* is definitely poor.

It must be emphasized that there is no sharp differentiation of these four types. A fungicide theoretically may have any slope value and any value of LD-95, so long as they are positive. The types discussed simply represent the extremes of behavior.

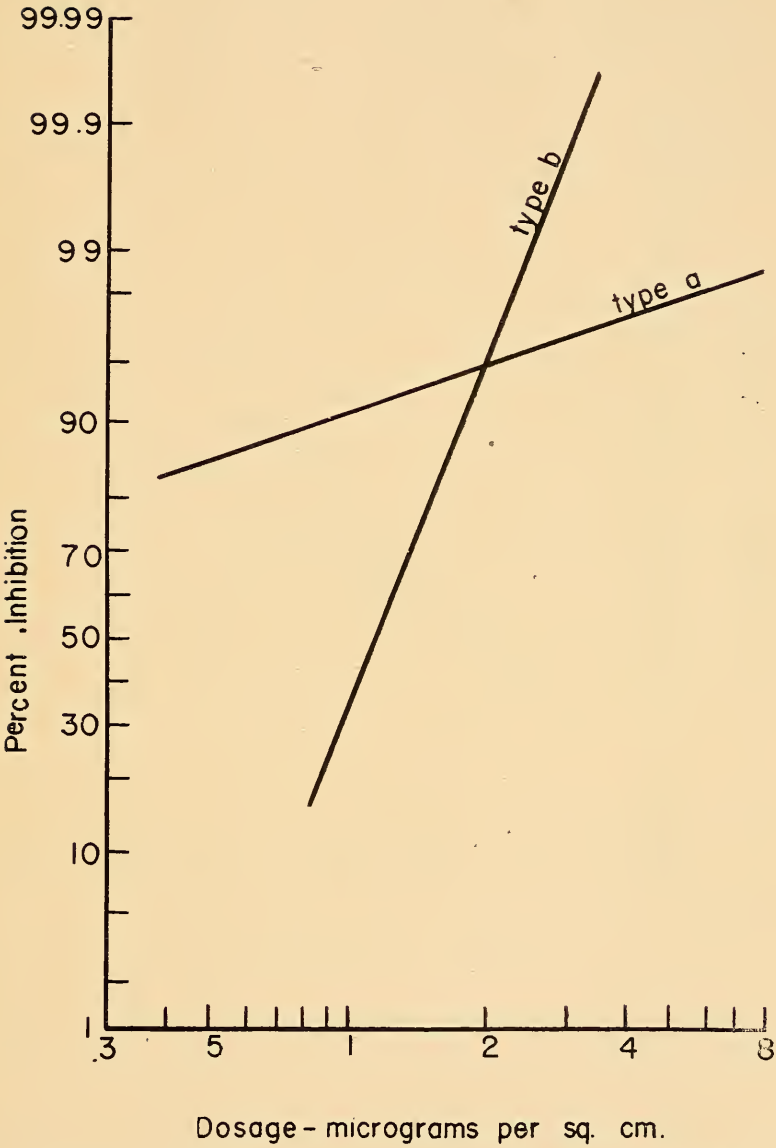

FIGURE 7. The dosage-response curves of two useful types of fungicides.

If no sharp differentiation can be made between the four types noted above, fungicides may be, and commonly are, encountered which have similar LD-95 values and different slopes, or similar slopes and different LD-95 values. The former possibility gives rise to an interesting phenomenon.

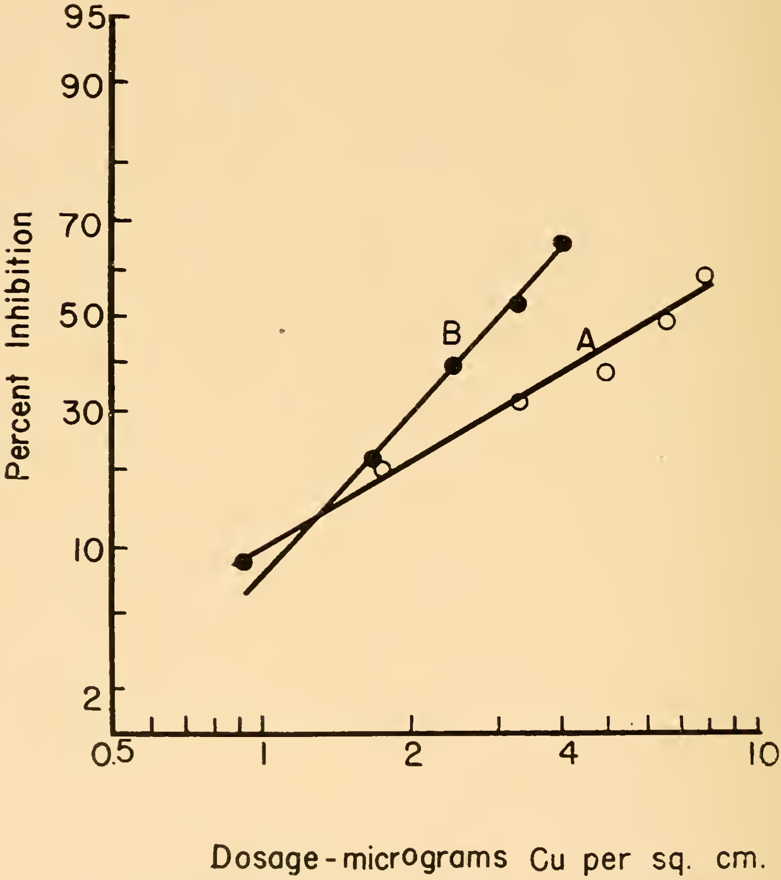

FIGURE 8. Dosage-response curves for tribasic copper sulfate (curve A) and for copper zeolite (curve B), illustrating the reversal effect from laboratory data.

If the dosage-response curves for two toxicants show similar LD values and different slopes, the curves must cross each other. Dosage-response curves for copper zeolite and tribasic copper sulfate illustrate this phenomenon (Figure 8). At the intersection point, these two materials have identical spore-inhibiting power, and in this case, the LD-15 is identical for the two materials. Below the intersection point, the toxicant having the flatter slope (tribasic copper

sulfate) produces greater inhibition per unit of dosage than the material with the steeper slope (copper zeolite), while above the intersection point, the order of effectiveness is reversed. Consequently, if the dosage-response curves of two toxicants cross one another, the *evaluation which* these *materials receive depends upon the level of inhibition under consideration.* In theory, all dosage-response curves cross each other at some level of inhibition unless they are parallel. This phenomenon and the conclusion drawn from it, takes on great importance when the corresponding condition is encountered in the field.

DOSAGE-RESPONSE CURVES CONSISTING OF BROKEN LINES

From time to time toxicants are encountered for which the dosage-response curve is not a straight line on logarithmic-probability coordinates. These appear to be exceptions to the general rule, however. McCallan, Wellman, and Wilcoxon (17) have observed dosage-response curves consisting of broken lines in the case of a number of inorganic salts, but have not attempted to explain the phenomenon. No more than 15 percent of the compounds which have been tested show a departure from linearity.

The existence of curves consisting of broken lines is of very far-reaching theoretical significance, for, if toxicity curves are ever anything but linear, it becomes dangerous to extrapolate from a series of experimental points. This is true in any case, and it should be a matter of general practice that toxicants proving promising in the laboratory should be tested throughout the entire range of toxicity. Extrapolated values from later experiments then are not dependent upon the assumption that the toxicant is effective through one mechanism of toxic action over its entire range.

DOSAGE-RESPONSE CURVES EXHIBITING PEAKS

Occasionally toxicants are encountered having dosage-response curves that cannot be resolved to straight lines over any extended range of inhibition. This type of spore inhibition is most clearly manifest when the toxicant is finely dispersed or dissolved in water and applied with the proper spore load directly to coated glass slides. The toxicant is never permitted to dry down and redissolve in such a case.

A series of experiments investigating this type of behavior was conducted, using tetramethylthiuram disulfide as toxicant and *Macrosporium sarcinaeforme* as test organism (Figure 9). This curve is of markedly different nature from those previously encountered, for it shows three distinct regions of toxic activity. In such cases, the toxicant exhibits a range of concentrations in water for which there is almost linear decrease[1] in toxicity with dosage on the logarithmic-

[1]In all of the foregoing discussion, the approach has been through the effect of *increasing* dosage upon the level of spore inhibition effected. In this particular case, it is necessary for the sake of simplicity in presentation of the argument to shift the approach, and to consider the effect of a *decrease* in dosage on the level of spore inhibition effected. This is necessary, inasmuch as the classical approach to dissociation and association of compounds is through the effect of increasing dilution, *i.e.*, decreasing concentration, upon the degree of dissociation of the compounds involved.

probability scale. At some point along this range, the toxicity increases with further dilution, and finally toxicity again falls with dilution.

It is to be noted that there are two distinct regions of the dosage-response curve for which dosage and response bear linear relation to one another. In each of these regions the toxicant has characteristic LD-50 and slope values. Toxicologists explain shifts in

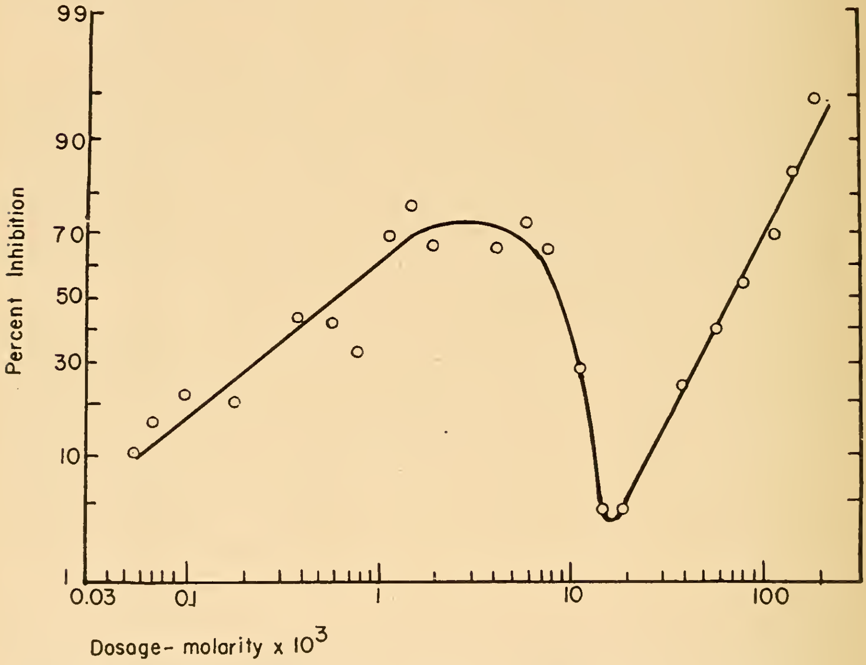

FIGURE 9. The dosage-response curve of tetramethylthiuram disulfide, showing a peak of toxic action.

slope of the dosage-response curve by postulating a shift in the mode of toxic action. It falls to us to answer two distinct questions in accounting for the behavior of tetramethylthiuram disulfide: (1) How can a single toxic agent pass through two distinct dosage regions, each with its characteristic LD-50 and slope values? (2) How can a material become more toxic on dilution?

A possible explanation of this type of behavior is that dissociation[1] or association of the toxicant occurs. It seems likely that weakly-dissociating materials may dissociate (or associate), forming

[1]Chemically, two types of dissociation appear to occur. Many inorganic salts are thought of as being completely dissociated at all times, even in the crystalline state. Organic compounds, and the weakly-dissociating, inorganic compounds are visualized as being undissociated or only very weakly dissociated in the crystalline state and in concentrated solution, but they become more and more dissociated on dilution, and at infinite dilution are thought of as being completely dissociated.

a complex which has toxicity markedly different from the original molecule. In the case of tetramethylthiuram disulfide, the toxicity of the dissociation complex would be greater than that of the undissociated molecule.

In the first phase of toxic action, where decrease in toxicity is proportional to dilution on the logarithmic-probability scale, the proportion of dissociated molecules as compared with the dissolved, undissociated molecules of toxicant might be very small, and toxic action would be determined largely by the undissociated molecules. As dilution continues, however, this proportion of dissociated to undissociated molecules increases, and, if the dissociated molecule is very much more toxic than the undissociated molecule, the inhibition of spores will rise with further dilution. Finally, when the original toxicant is completely dissolved and dilution has progressed to the stage at which there are no undissociated molecules left, the second peak of toxicity has been reached. Dilution beyond this point can only cause a decrease in concentration of dissociated molecules and in toxicity.

If the offered hypothesis proves to be true, one may predict that materials may be encountered for which the maximum of the second peak of toxicity is very high or very low and in which the second peak is very widely separated from or very close to the initial peak of toxicity on the dosage scale. The factors governing these properties would be the ratio of toxicity of the undissociated (or unassociated) to the dissociated (or associated) molecules, and the rate at which the dissociation occurs both with respect to time and to the addition of further solvent.

In contrast is the possibility that the toxicant may dissociate (or associate) and that the dissociated molecule is very much less toxic than the undissociated molecule. In this case, the opposite type of action would take place. The initial slope of the toxicity curve would be flatter than the second phase of toxicity.

These possibilities may well serve to explain the types of broken lines observed by McCallan, Wellman, and Wilcoxon (17). The concave type of broken line observed by them would be the result of dissociation (or association) in which the dissociated molecule is more toxic than the undissociated molecule, whereas the convex type of curve would result from the other possibility; *viz.*, when the dissociated (or associated) molecule is much less toxic than the original molecule.

The postulated mode of action could explain these broken lines only if it is found that there is a smooth transition from one linear mode of toxic action to another, rather than the sharply broken lines described by them (17). As stated previously a shift in slope of the toxicity curve is presumptive evidence for a shift in the mode of

toxic action. On the assumption that a salt can kill spores by more than one distinct mechanism, it is difficult to explain why the transition should be so sharp as these workers have described it. Their interpretation of their data indicates that toxic action must operate by one mechanism or by another without any intermediate stages. One would expect on *a priori* grounds that stages of transition would be encountered. Bliss (3) has considered the case in which insecticides act jointly and has presented hypothetical curves which show such a transition. As a matter of fact, the experimental points for two figures presented by McCallan, Wellman, and Wilcoxon (their figures 1c and 2a) (17) show evidence quite clearly for a smooth transition. If the dissociation (or association) mechanism offered above is valid in this case, the factor governing the mode of toxic action is the ratio of one type of molecule to another, and this ratio must increase gradually with dilution. This being the case, one would expect that toxicants acting in accordance with such a mechanism will show a smooth transition between modes of action, and it is felt that a critical examination of this point would prove of interest.

If water enters into this postulated process, such behavior would be a type of synergistic action. Synergism is defined as the action of two or more toxicants in conjunction with one another in such manner that the total toxicity is greater than the sum of the two toxicants acting in that dosage independently. Although water can hardly be considered a toxicant under ordinary circumstances, it may be so considered in such a case.

Clark (5) observed a type of toxic action falling into this category in a study of the combined toxic action of $HgCl_2$ and various alkali chlorides upon fungous spores. When NaCl was added to $HgCl_2$ in progressive amounts, toxicity increased until the molecular ratio of $HgCl_2$ to NaCl reached the value of 1:3600 and then toxicity fell beyond this point. Clark suggested that the excess of Cl^- ion supplied by NaCl caused the formation of $HgCl_4^{--}$ ions which were supposed to be more toxic than $HgCl_2$ itself, and that the peak of toxicity coincided with the maximum concentration of $HgCl_4^{--}$ ion. The fall beyond this point was ascribed to the formation of the relatively non-toxic double salt Na_2HgCl_4.

Clearly, the case observed by Clark is a type of synergism, commonly observed by now, in which two metallic salts act to form an ion and finally a double salt, each of which has toxic properties differing markedly from those of the original components.

The case of the tetramethylthiuram disulfide differs only in that a single molecular species is introduced into the aqueous phase, and that either dissociation or association occurs, with or without water molecules entering into the process. The result of this action is that a complex is formed, having different toxicity from that of the original, undissociated molecule.

EVALUATION OF FUNGICIDES IN THE FIELD

Properties of a Good Protective Fungicide

It would be well at this point to assemble our information and to define some of the properties which a good protective fungicide must have. A good fungicide, in addition to the obvious properties of availability, low cost, lack of phytocidal action, ability to remain active over extended time periods, and freedom from toxicity to man or animals, should have the following fundamental properties: (1) Above all else, its LD-95 must be low. (2) If the dosage-response curve has a steep slope, the tenacity for the fungicide must be high. Bordeaux mixture, for example, has a very steep slope and is effective as a protectant because it has an exceedingly high tenacity and a low LD-95. (3) If the dosage-response curve is flat, high tenacity is desirable but not essential. No such fungicide is in field use at the present time, and tests searching for a material better in performance than Bordeaux mixture might well have as an objective the finding of a material with these properties.

Field Evaluations Involving Single Fungicides

Experience gained through laboratory tests has pointed out a number of important factors which influence the performance of a material as a fungicide. On the assumption that factors in the laboratory are homologous (15) with those in the field, it is of interest to see to what extent these factors or their field homologues affect protective value in the field.

LINEAR DOSAGE-CONTROL CURVES IN THE FIELD

In the past, no simple relation has been found between amount of disease and dosage of fungicide in the field, probably because the pathologist has concerned himself with such high degrees of disease control that it has been difficult to measure the relationship between these factors. If the laboratory and field are homologous, the factor corresponding to spore-inhibition in the laboratory is disease control in the field.[1] It follows that fungicides with linear dosage-response curves in the laboratory should likewise have linear dosage-control curves in the field, when data are plotted on logarithmic-probability coordinates.

To test whether or not this is the case, several field experiments were conducted in which a series of dosages of each fungicide was applied. In all cases, disease control showed linear relation to dosage of fungicide (Figure 10). In one case, the control of apple scab by a series of dosages of a wettable sulfur was tested. Foliage and fruit readings showed a similar relationship of disease control to dosage of sulfur. The control of *Alternaria* blight on tomatoes by a series of dosages of yellow cuprous oxide was tested also. The

[1]The performance of a protective fungicide in controlling disease in the field directly involves the tenacity of a fungicide. Accordingly, the weathering behavior of a fungicide is superimposed upon the spore-inhibiting properties under field conditions. Experiments testing the nature of this relation are now in progress.

relation found for the control of *Septoria* blight on celery, when a series of dosages for an organic copper compound was applied, was confirmatory to the other two experiments.

The first three curves of Figure 10 are for foliage diseases. The fourth (curve D) shows the relation of dosage to control for a seed-borne disease: wheat bunt by seed treatment with red cuprous

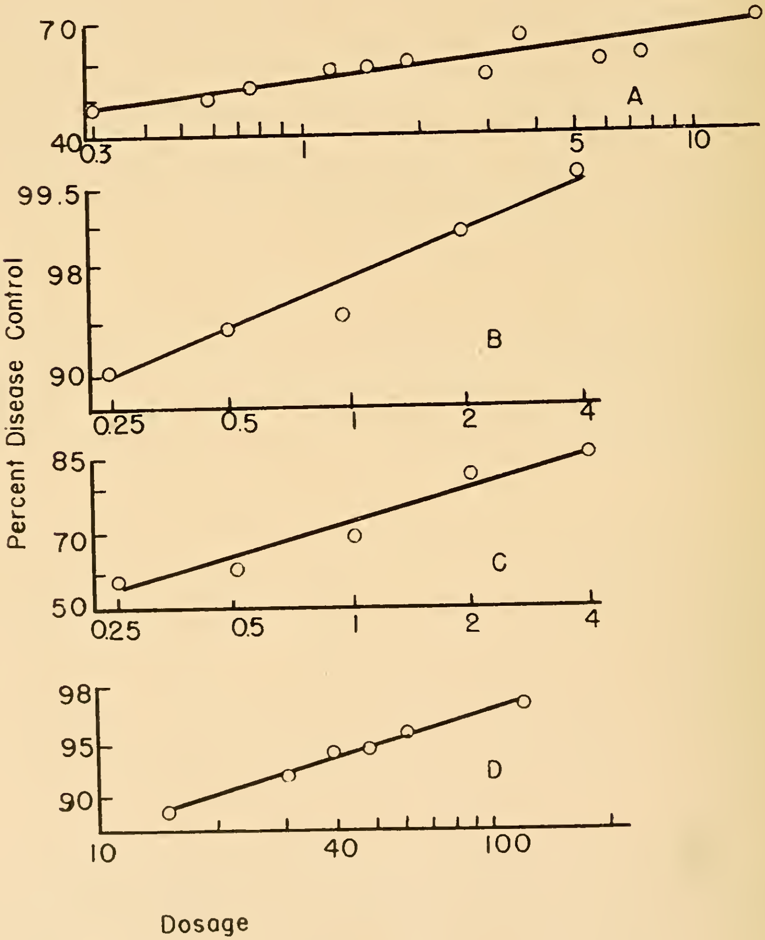

FIGURE 10. The linear relation in the field between dosage of fungicide and the percentage of disease control. Curve A: control of apple scab by sulfur. Curve B: control of *Alternaria* blight of tomatoes by yellow cuprous oxide. Curve C: control of *Septoria* blight of celery by an organic fungicide. Curve D: control of wheat bunt by red cuprous oxide. In curves A, B, and C, dosage is in units of pounds per 100 gallons. In curve D, dosage is in units of grams of fungicide per bushel of seed.

oxide.[1] Here amount of disease was determined by making counts of the smutted heads; disease control percentages are estimated directly.

It would appear (Figure 10) that a method is now at hand whereby dosage and control can be related by means of a simple line. There are great possibilities for use of this relation. For example, it is possible to interpolate values and to extrapolate within reason to determine the fungicide dosage which would have been necessary to give a desired level of disease control. Such data are of little use in making predictions at the present time, for, as will be shown, uncontrollable environmental factors affect the properties of these lines from one experiment to the next.

PROPERTIES OF THE LINEAR DOSAGE-CONTROL CURVE

(A) **LD-95.** It is of interest to see how the factors that affect LD-95 and slope in the laboratory affect them in the field. In the laboratory, the fungous species used, the fungicide itself, spore load, and slope of the dosage-response curve are factors that influence the value of the LD-95. In the field, these same factors determine the value of the LD-95, and they exert their influence in the same way. The first two of these factors are matters of common experience. It is common knowledge that a given fungicide is not equally effective against all fungi attacking a given crop, and that fungicides differ in their ability to control a given disease.

The influence of spore load on the LD-95 of a fungicide under field conditions can be demonstrated from the data of Twentyman on wheat bunt (20). He divided a wheat seed sample into three lots and artificially inoculated these seeds with bunt at three spore loads: 1.0 percent, 0.4 percent, and 0.1 percent by weight. He then applied copper carbonate to each of these seed lots, in a dosage series. Figure 11 shows the effect of spore load on the dosage-control curve when logarithmic-probability coordinates are used. It can be seen that, just as in the laboratory (Figure 6), increasing the spore load displaces the dosage-control curve to the right proportionately with the increase in spore load, logarithm of spore load increasing proportionately with the logarithm of the dosage. Again, as in the laboratory, spore load has no effect on the slope of the dosage-control curve.

In most disease control research it is seldom possible to control or to predict spore load. If either of these could be done, the prediction of the quantity of fungicide necessary to give a desired level of control would be greatly simplified. The number of spores caught in the field in spore traps gives an approximation to the actual spore load. Experiments testing the effectiveness of this method of arriving at spore load are now in progress. The recently published report

[1]The data utilized to construct curve D of Figure 10 are the unpublished results of experiments, conducted by J. G. Horsfall and Robert Magie at the New York State Agricultural Experiment Station in Geneva.

of the Committee on Aerobiology (6) suggests a number of techniques which will greatly simplify the task of arriving at a suitable method for an estimation of spore load.

(B) **Slope.** Different fungous species may show characteristic slopes for dosage-control curves for one fungicide, although this point needs further investigation. Any environmental factors which

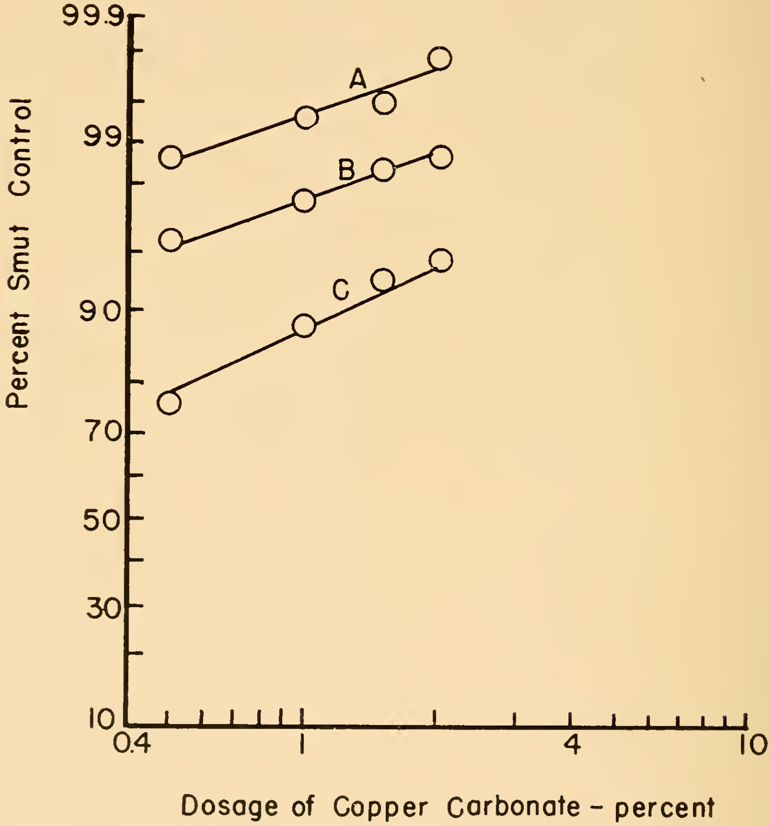

FIGURE 11. The relation of spore load to LD-95 in the field. Control of wheat bunt by copper carbonate. Data of Twentyman (20). Curve A: 0.1 percent by weight of smut. Curve B: 0.4 percent smut. Curve C: 1.0 percent smut.

favor the development of the fungus and raise its chances of infecting the host, appear to affect the slope of the dosage-control curve in the field as they do in the laboratory.

It is particularly difficult to design an experiment to test this point, for the investigator usually is forced to accept the environmental conditions which prevail in the field while an experiment is

in progress. During 1940, a series of sulfur dosages was applied to McIntosh apple trees to measure the properties of dosage-control curves for apple scab. Two readings of scab incidence on foliage were made during the season: one late in June and one late in July (Figure 12). During June conditions were rather unfavorable for scab development, whereas during July weather conditions were much more favorable. The dosage-control curve obtained in June

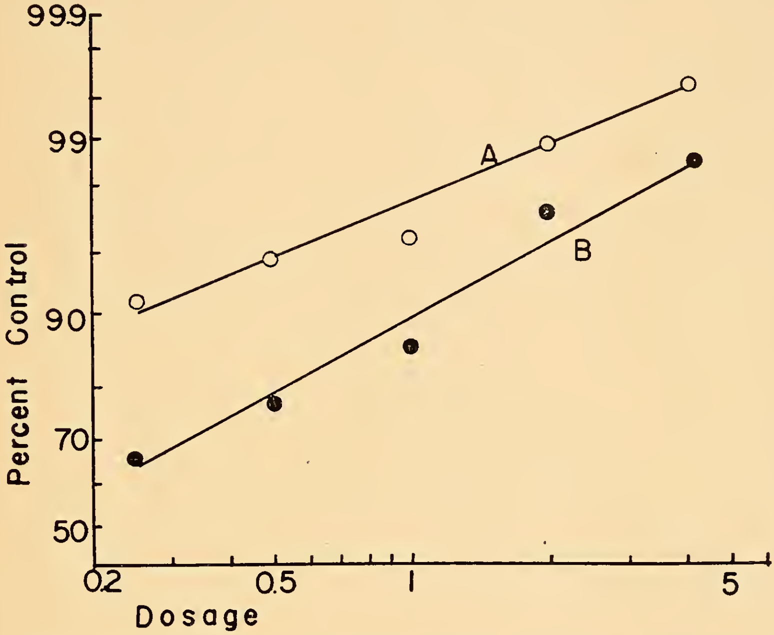

FIGURE 12. The relation between environmental conditions affecting disease development and slope of the dosage-control curve for apple scab. McIntosh apple trees were sprayed with sulfur. Data for curve A were obtained on June 20 when conditions for scab development were only fair. Data for curve 2 were obtained July 24 when conditions for scab development had been much better.

is much flatter than the one obtained in July, indicating that under field conditions, too, environmental conditions which favor the development of the fungus increase the slope of the dosage-control curve.

This conclusion has been confirmed twice during the field season of 1941, in one case on apples for scab, and in another on tomatoes sprayed with a number of dosages each of red cuprous oxide, Bordeaux mixture, and copper oxychloride (Compound A).

Coverage of the plant by fungicide likewise affects the slope of the dosage-control curve. In the past, what has been called cover-

age has been measured by chemical as well as by biological means, but the designs of the tests have been such as to give information only concerning how much fungicide was present over the leaf surface. This is really a measure of dosage. Coverage is a measure of the distribution of fungicide over the surface of the plant as a whole, the inside, unprotected leaves as well as the outer, exposed ones.

The technique to be described gives a biological measure of coverage. If several concentrations of fungicide are applied to plants,

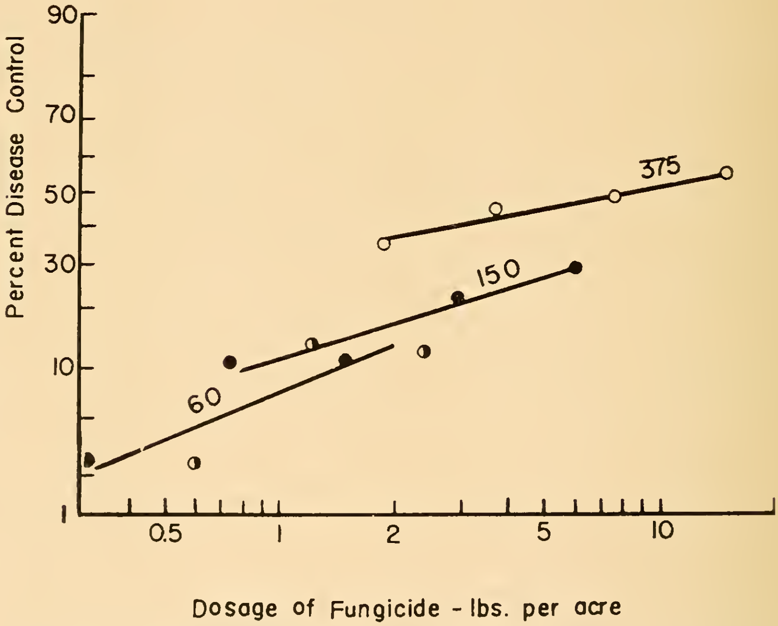

FIGURE 13. The coverage effect, showing the relation between the number of gallons of spray applied per acre and the slope of the dosage-control curve. Yellow cuprous oxide applied to tomatoes for the control of *Alternaria* blight. Numbers on curves denote gallonage of spray applied per acre.

and if each of these concentrations is applied at several gallonages,[1] the effect of coverage on disease control can be determined very readily. During the summer of 1940, an experiment of such design was carried out,[2] using yellow cuprous oxide on tomatoes for the control of *Alternaria* leaf spot (7). Four concentrations: 2, 1, 0.5, and 0.25 pounds of copper per 100 gallons were employed. Each concentration was applied at the rate of 60, of 150, and of 375 gal-

[1]Gallonage may be varied by changing (1) rate of spray output and (2) the time of spraying, with constant rate of spray output. In these experiments, nozzles of successively larger aperture were employed to increase gallonage of spray.

[2]We are indebted to Mr. A. D. McDonnell, research assistant, who sprayed these plots.

lons per acre to give three degrees of coverage. In all, 12 types of spray treatment were applied, together with the check. When data as percentage of disease control were plotted against the dosage of fungicide applied per acre (concentration times gallonage), three curves were obtained (Figure 13), one for each of the three gallonages applied. As the coverage was improved by higher gallonage, the slope became flatter. From this it follows that the slope of the dosage-control curve can be a measure of the coverage of the plant by fungicide. This conclusion was confirmed by plotting the slope values arithmetically against the gallonage of spray applied per acre to obtain a single straight line function (Figure 14).

That the slope of the dosage-control curve becomes flatter at a higher rate of spray application merely indicates that the fungicide becomes so efficiently spread over the leaf surface of the plant as a whole that it makes little difference whether moderate or high dosage of fungicide is applied (7). It will be seen at the same time that the LD values for fungicide have been very materially reduced.

Any factor which tends to reduce the LD value and/or decrease the slope of a fungicide at the same time can and should be used so as to increase the efficiency of a fungicide.

USE OF LD-95 AND SLOPE TO MEASURE DYNAMICS OF DISEASE DEVELOPMENT

The LD-95 and the slope of the dosage-control curve offer the investigator a new approach to the study of disease development, host-parasite relations, virulence, disease-resistance, etc. The LD-95 and the slope of the dosage-control curve have been shown to be dependent for their particular values in an experiment upon the fungicide, the fungus, the spore load of the fungus, and the environmental conditions. This being true, LD-95 and slope of the dosage-control curve can be used in quantitatively measuring the host-parasite relationship as influenced by the fungus, the spore load, and the environment. To do this, it is necessary to employ a single standard fungicide in tests, and to hold constant all factors which alter LD-95 and slope other than the factor being studied. It has been shown that spore load exerts its effect primarily through shifts in the value of the LD-95 (and LD-50). As has been pointed out, LD-95 values will give a more accurate measure of shifts of the fungus load than LD-50, since variations in LD values due to slope variations are at a minimum in this region.

Apparently we have at hand a means of separating out factors tending to produce disease attributable on the one hand to the fungus, and on the other to the environment. Factors dependent upon the fungus would reflect their action through the LD-95 values; *i.e.*, dosage-control curves would be displaced to the right or left without a shift in slope, whereas the influence of climatic factors could be estimated through the magnitude of the shift in slope of the dosage-control curve.

Evaluations Involving Two or More Fungicides

THE REVERSAL EFFECT

The discussion immediately preceding was limited to the simple case of studies involving single fungicides. What has been said there applies equally well in the case of comparisons of two or more fungicides in the same test. The picture is a little more complex.

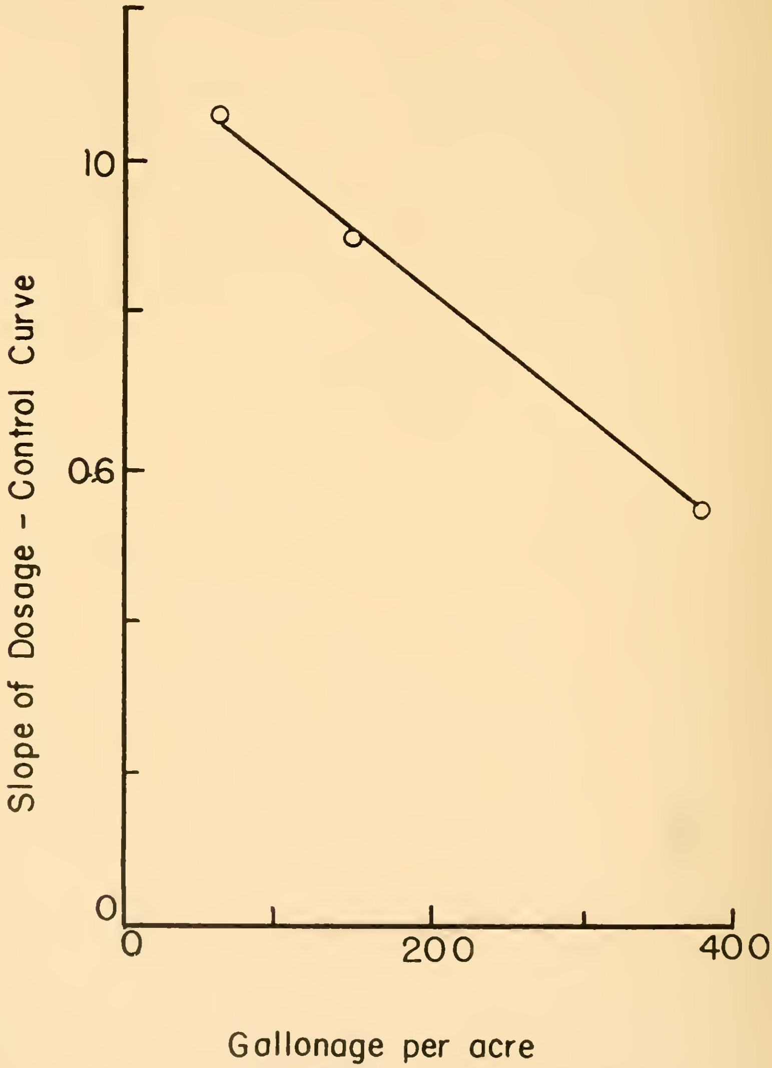

FIGURE 14. The relation between the rate of increase of plant protection and the number of gallons of spray applied per acre.

however, because the ranking of a series of fungicides often changes from test to test, from year to year, or from state to state. Such conflicting results cannot be dismissed easily.

A study of the types of dosage-control curves affords an explanation for many instances of this kind. Dosage-control curves for damping-off of spinach in the field were obtained for red cuprous oxide and for zinc oxide, during the past year (Figure 15). The curve for zinc oxide is much flatter than for cuprous oxide. This situation (Figure 15) is strictly homologous with that already observed in the laboratory (Figure 8). At the intersection point, the two materials are equally effective. Below the intersection point, *i.e.*, for levels of control below 62 percent, it may be expected that a given dosage of zinc oxide will give a higher degree of control

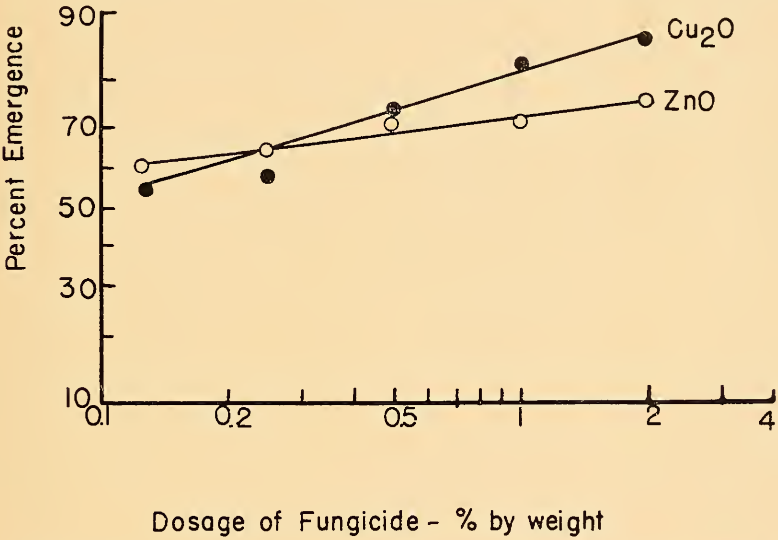

FIGURE 15. The reversal effect, where the dosage-control curves for two fungicides (red cuprous oxide and zinc oxide) cross each other, with resultant reversal in order of effectiveness of toxic action. Curves are for seed protection against damping-off of spinach.

than will cuprous oxide. Above the intersection point, it is apparent for the experimental conditions that, for the equal dosages of the two fungicides, red cuprous oxide will give the higher level of disease control. That changed environmental factors will cause a shift in slope of the dosage-control curves has already been emphasized. If, in one test, environmental conditions were such that the investigator, dealing with single dosages of each fungicide, happened to compare them above the intersection point of their dosage-control curves, and, in the second test, environmental conditions were different so that this time the investigator compared the same materials below the intersection point of their dosage control curves, one would expect that the order of effectiveness of the two materials would be reversed.

Experiments are under way which are designed to test whether the shift in slope for two fungicides must be identical in all cases or whether the shift due to environmental factors may be greater for one fungicide than for another; *i.e.*, whether slopes may shift differentially.

Figure 15 demonstrates a second important point. Since the relative value of two or more fungicides with dosage-control curves which are not parallel becomes more widely divergent, the farther away from the intersection point one makes the comparison, it is apparent that *the relative effectiveness of members of a series of fungicides depends upon the level of disease control under consideration.* It also follows that *the level of disease control at which fungicides are compared must be explicitly stated.*

COMPARISON OF FUNGICIDES BY MEANS OF THE DOSAGE REQUIRED FOR EQUAL LEVELS OF DISEASE CONTROL

In the past it has been the practice to apply fungicides under test at a single dosage each, and to rate the fungicides on the basis of the level of disease control obtained. In the foregoing discussion it has been shown that control-for-equivalent-dosage only gives a picture of the relative value at one fungicidal dosage and nothing is learned of the relative performance at different dosages.

A more informative measure of the relative value of a series of fungicides is to compare them at a series of dosages and to rate them by means of the dosage required for each material to effect the same level of disease control. This manner of comparing fungicides has the advantage that materials are compared at levels of similar physiological effect. Comparison of the control obtained from equal dosages presumes that the differences between fungicides can be estimated from differences in level of control obtained. This is definitely not the case. Figure 16 will illustrate this point.

In this figure are drawn curves for two fungicides. If the fungicides were applied at a dosage of 2 pounds per 100 gallons, fungicide A would show a level of 90 percent control, while fungicide B would show a level of 50 percent control. The practice has been to state that, since one fungicide gives 90 percent control and the other gives only 50 percent control, A is about twice as effective as B. However, if the fungicides had been applied at a concentration of 4 pounds per 100 gallons, fungicide A would have shown 95 percent control, whereas fungicide B would have shown 80 percent control, and would now be only about 1.2 times as effective as B. Moreover, this method of making the comparison has led to a false conclusion, since, in either case, if the dosage of fungicide B is increased to the extent by which it is thought to be inferior to fungicide A, it will still be inferior to A, and the ratio comparing the two, therefore, has not yielded the information desired.

A comparison of fungicides through the dosage to effect equal levels of disease control would have shown that, at a level of 90 percent disease control, 2 pounds per 100 gallons of fungicide A were required, whereas 6 pounds per 100 gallons of fungicide B were needed. Therefore, for that level of disease control, A is three times as effective as B. This method of making the comparison is more sensitive and more informative than the old one.

Comparing fungicides through the dosage for equal control does not tell the whole story, however, for this comparison is through

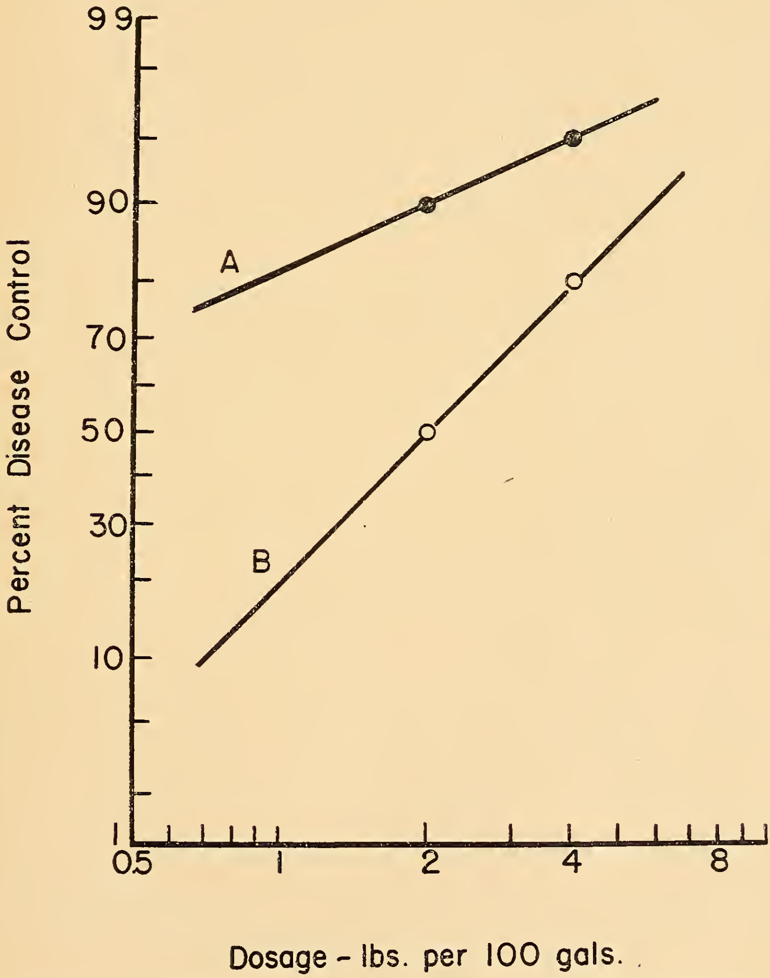

FIGURE 16. Hypothetical dosage-control curves for two fungicides, illustrating the advantages of comparing them through the dosage required to effect equal levels of disease control.

LD values only. Just as it is desirable in the laboratory to make use of slope as a factor in the rating of fungicides, so is there the need in the field that slope of the dosage-control curve be used as an index of fungicidal value.

Probably no one index can be taken as a completely satisfactory measure of both slope and LD-95, either in the field or in the laboratory, particularly since the importance of slope and tenacity are so intimately inter-related. The weighting of these factors by a number measuring their relative importance under field conditions is an impractical and probably useless task, since in the dry season tenacity is less important than in a wet one, and since the requirements for the control of different types of fungi vary so widely.

SUMMARY AND CONCLUSIONS

The testing of fungicides in the laboratory has given rise to a number of new concepts, some of which are presented in this paper:

1. Field experiments in which fungicides are tested can profitably be patterned more closely after the design of laboratory experiments in current use for the testing of fungicides.

2. In the laboratory a fungicide is tested at a number of dosages, and data are obtained on the spore inhibition which these dosages effect. The plotting of these dosage-response data on a logarithmic-probability grid generally yields straight lines (22).

3. There are two notable exceptions to the type of toxic action which yields straight lines on logarithmic-probability paper. McCallan, Wellman, and Wilcoxon (17) have reported toxic action in which the dosage-response curve consists of two or more linear segments, the whole curve constituting a broken line. The present paper reports the existence of dosage-response curves exhibiting peaks of toxic action. Both of these types of dosage-response curves can be explained by the hypothesis that the toxicant dissociates (or associates) in water and that the dissociated molecule has toxicity differing markedly from that of the undissociated molecule.

4. Fundamental properties of fungicides derivable from the linear type of dosage-response curve are the LD-95 (or LD-50) *i.e.*, the lethal dosage for 95 (or 50) percent of the treated spore population, and the slope of the dosage-response curve. At the present time fungicides are evaluated in terms of the LD-95 (or LD-50) only; slope values should be included in the evaluation. LD-95 values are affected by the fungicide, the fungous species, age of the culture, and spore load used in the test, as well as by slope itself. Slope may be affected by the fungus used in the test and is increased by any environmental factor which tends to bring the fungus nearer its optimum. It is also a property of the fungicide when all other factors are held constant.

5. The slope of the dosage-response curve of a fungicide gives an indication of the importance of tenacity to the material. In gen-

eral, fungicides performing under such conditions that their dosage-response (or dosage-control) curves are flat need not have such high tenacity values as when the slope of these curves is steep.

6. Properties of a good fungicide include low LD-95 (and LD-50), flat dosage-control (and dosage-response) curves, or if this curve is steep, high tenacity.

7. The assumption has been made that there is an homology between laboratory and field conditions for fungicide tests:

a. This hypothesis is supported by the fact that, when a fungicide exhibits linear dosage-response curves in the laboratory, the dosage-control curve in the field is also linear.

b. Also supporting this hypothesis is the fact that spore load and environment influence the values of LD-95 (and LD-50) and slope of the dosage-control curves in the same way that they affect dosage-response curves in the laboratory.

8. It has been shown that the efficiency of fungicidal action may be markedly improved by more effective coverage of the plant by fungicide.

9. Often in repeated tests involving two fungicides, one material will perform better in some cases and will be inferior in others. This anomaly is explained by the reversal effect as being due to the crossing-over of dosage-control curves and to the differential shift in slope of these curves with changing environment.

10. It is suggested that in field tests, fungicides may be more quantitatively compared by measuring the dosage of each required to effect the same level of disease control, rather than by measuring the levels of disease control obtained through the application of single fungicide dosages. This involves applying each fungicide in a dosage series.

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